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Data in Brief

Transcriptome analysis of T_H2 $CD4^+$ T cells differentiated from wild-type and NLRP3KO mice

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ABSTRACT

The Nod-like receptor NLRP3 is involved in the formation of NLRP3. Up to now, the immunological functions of NLRP3 independently of inflammasome is unclear. In this dataset containing 6 samples (T_H0, T_H2 cells at day 3 and day 6 in wild type or *Nlrp3* deficient cells), we show that NLRP3 expression in $CD4^+$ T cells supports a T helper 2 (T_H2) transcriptional program in a cell-intrinsic manner (raw and normalized data are accessible on Gene Expression Omnibus database under the number GSE54561, http://www.dtd.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54561). Indeed, NLRP3 positively-regulated T_H2 program independently of inflammasome formation. These data indicated that T_H2 specific genes such as cMaf or Il4 were not induced in Nlrp3 deficient cells. These results demonstrate the capacity of NLRP3 to act as a key transcription factor in T_H2 differentiation.

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Specifications	
Organism/cell line/tissue	*Mus musculus*
Sex	Sex independent
Sequencer or array type	MiSeq (Illumina)
Data format	Raw data and normalized data
Experimental factors	T CD4 lymphocytes obtained from mice and differentiated in Th2 subtype
Experimental features	The transcriptional profile of T CD4 lymphocytes obtained from wild-type or NLRP3KO mice differentiated in Th2 cells was analyzed by RNA sequencing.
Consent	Protocol approved by ethic committee
Sample source location	France

1. Direct link to deposited data

http://www.dtd.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54561.

2. Experimental design, materials and methods

The transcriptome of $CD4^+$ T cells was studied by RNA sequencing on MiSeq device (Illumina). Naive $CD4^+$ T cells ($CD4^+CD62L^{high}$) from spleens and lymph nodes of C57BL/6 Nlrp3 wild type or *Nlrp3*$^{-/-}$ mice were sorted by flow cytometry with BD ARIA cytometer equipped with BD FACSDiva software (BD Biosciences). The purity of isolated naive T cell population exceeded 95%. Naive T cells were cultured for three days with anti-CD3 (2 μg/mL) and anti-CD28 (2 μg/mL) antibodies in the presence of anti-IFNγ (10 μg/mL) and anti-IL-4 (10 μg/mL) to obtain T_H0 or of anti-IL-4 (10 μg/mL) and IL-12 (10 ng/mL) to obtain T_H1 or of anti-IFNγ (10 μg/mL) and IL-4 (10 ng/mL) to obtain T_H2. Cells were cultured at 37 °C under 5% CO_2 in RPMI 1640 with 5% (vol/vol) fetal calf serum supplemented with sodium pyruvate, penicillin and streptomycin, and 4 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Anti-CD3 (clone 145-2C11), anti-CD28 (clone PV-1), anti-IL-4 (clone 1B11) and anti-IFN-γ (clone XMG.1) antibodies were obtained from BioXcell and IL-12 and IL-4 were purchased from R&D Systems. A second round of stimulation was done using the same cytokine cocktails plus IL-2 (100 U/mL, R&D Systems) for another three days.

For RNA-Seq library preparation, total RNA from T cells was extracted using TRIzol (Life Technologies). rRNA was removed, thanks to Ribominus Kit (Life Technologies). 100 ng of rRNA depleted RNA was used for the library preparation, thanks to the NEBNext Ultra RNA library kit for Illumina (New England Biolabs) by following the manufacturer's instructions. Briefly, RNA was heat fragmented and a reverse transcription was performed. Next, the second strand of cDNA was synthesized and the reaction mixture was purified with magnetic beads. Afterwards, an end repair step was carried out and adapters were ligated. After second magnetic bead purification, a PCR of 12 cycles was performed to increase the target yield and incorporate index. Quality and quantity of libraries were assessed after magnetic bead purification thanks to a TapeStation fragment analyzer (Agilent). Library size was

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between 250 base pairs and 400 base pairs. Each library was diluted at a concentration of 4 nM. Five libraries were pooled and 12 pM of the mix was injected into the sequencing flow cell. The RNA-Seq libraries were sequenced with paired-end 151 bp reads. Data analysis was carried out via Galaxy interface [1]. FASTQ files were mapped by using BWA (mm9 version of *Mus musculus* genome) for Illumina [2]. The analysis was performed by using TopHat for Illumina [3]. Generated files were processed with Cufflinks software [4] to obtain annotated expressed genes in each studied subtype. Then, differential expression between the samples was analyzed with Cuffdiff [4]. Raw and normalized data were accessible on public database: GEO submission number GSE54561, http://www.dtd.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54561 [5].

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